

*cn:t*  
*A*  
*8*  
Exemplary peptide sequences are:

RRHTRSAEDDSERD (SEQ ID NO: 19) (residues 175-189 of SEQ ID NO:4) and  
YHLQIHKNGHVDGAPHQ (SEQ ID NO: 20) (residues 51-67 of SEQ ID NO:4).

In the claims:

*Sub B3*  
17. The epitope-bearing portion of claim 15, which comprises amino acids  
RRHTRSAEDDSERD (SEQ ID NO: 19).

*Aq*  
18. The epitope-bearing portion of claim 15, which comprises amino acids  
YHLQIHKNGHVDGAPHQ (SEQ ID NO: 20).

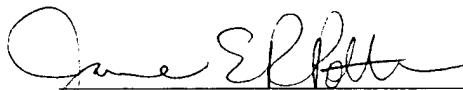
REMARKS

The enclosed electronic and paper copies of the Sequence Listing include no new matter that goes beyond the original application as filed, but are supplied to fulfill the requirements as outlined in the Notice to File Missing Parts. Furthermore, the above amendments, which merely direct the insertion of the Sequence Listing and insertion of sequence identifiers, include no matter that goes beyond the original application as filed. Applicant respectfully submits that the above-identified application is now in compliance with 37 C.F.R. §§ 1.821-1.825.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The first of the attached pages is captioned "Version with Markings to Show Changes Made."

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Respectfully submitted,  
Seed Intellectual Property Law Group PLLC



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JEP:mls

Enclosures:

Computer Diskette  
Declaration Regarding Computer Diskette  
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Application No. : 09/801,968  
Docket No. : 17105.001/201130.40901

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

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In the Specification:

Paragraph beginning at page 4, line 16, has been amended as follows:

Figure 5. Amino acid sequence comparison of human FGF-23 (SEQ ID NO: 4) and human FGF-19 (SEQ ID NO: 14). Asterisks indicate identical amino acid residues of the sequences.

Paragraph beginning at page 4, line 18, has been amended as follows:

Figure 6. Amino acid sequence comparison of human FGF-23 (SEQ ID NO: 4) and human FGF-21 (SEQ ID NO: 15).

Paragraph beginning at page 5, line 20, has been amended as follows:

Figure 15. Figure 15 provides an alignment of members of the FGF family (SEQ ID Nos: 23-46), indicating putative cleavage sites for FGF-23.

Paragraph beginning at page 12, line 1, has been amended as follows:

Fusion proteins comprising FGF-23 or a biologically active or antigenic fragment thereof can be produced using methods known in the art. Such fusion proteins can be used therapeutically or can be produced in order to simplify the isolation and purification procedures. Histidine residues can be incorporated to allow immobilized metal affinity chromatography purification. Residues EQKLISEEDL (SEQ ID NO: 16) contain the antigenic determinant recognized by the myc monoclonal antibody and can be incorporated to allow myc monoclonal antibody-based affinity purification. A thrombin cleavage site can be incorporated to allow cleavage of the molecule at a chosen site; a preferred thrombin cleavage site consists of residues LVPRG (SEQ ID NO: 17). Purification of the molecule can be facilitated by incorporating a sequence, such as residues SAWRHPQFGG (SEQ ID NO: 18), which binds to paramagnetic streptavidin beads. Such embodiments are described in WO 97/25345, which is incorporated by reference.

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Paragraph beginning at page 30, line 19, has been amended as follows:

Oligopeptides can be selected as candidates for the production of an antibody to the FGF-23 protein based upon the oligopeptides lying in hydrophilic regions, which are thus likely to be exposed in the mature protein. Preferred oligopeptides are RRHTRSAEDDSERD (SEQ ID NO: 19) (residues 175-189 of SEQ ID NO:4) and YHLQIHKNGHVDGAPHQ (SEQ ID NO: 20) (residues 51-67 of SEQ ID NO:4). Additional oligopeptides can be determined using, for example, the Antigenicity Index of Welling, G.W. et al., FEBS Lett. 188:215-218, 1985, incorporated herein by reference.

Paragraph beginning at page 39, line 3, has been amended as follows:

*Isolation and Analysis of Mouse FGF-23.* DNA was amplified from mouse skin cDNA by adaptor-ligation mediated PCR using a Marathon cDNA amplification kit (Clontech) with the following primers: 5' ctgatgattacatcagaggac 3' (a sense primer for mouse FGF-23, SEQ ID NO:7); 5' caccaggtagtgtatgcttct 3' (an antisense primer for mouse FGF-23, SEQ ID NO:8); 5' atccatacaaaggAACCTcg 3' (an antisense primer for mouse FGF-23, SEQ ID NO:9); 5' ccatcctaatacgactcactatagggc 3' (an adaptor primer, SEQ ID NO:10); and 5'

actcactataggctcgagcggc 3' (an adaptor primer, SEQ ID NO:11). The cDNA encoding the entire coding region of the FGF was amplified by PCR using the primers 5' actcagtgcgtgcaatgct 3' (a sense primer for mouse FGF-23, SEQ ID NO: 12) and 5' gacctagacgaacctggaa 3' (an antisense primer for mouse FGF-23, SEQ ID NO: 13), and cloned into the pGEM-T DNA vector. The nucleotide sequence is shown in SEQ ID NO:1 and the amino acid sequence is shown in SEQ ID NO:2. The protein has 251 amino acids, including a signal sequence at approximately amino acid positions 1-24.

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Paragraph beginning at page 39, line 18, has been amended as follows:

*Production of Recombinant Mouse FGF-23 in High Five Insect Cells.* The mouse FGF-23 cDNA with a DNA fragment (75 bp) encoding an E tag (GAPVPYPDPLEPR) (SEQ ID NO: 21) and a His<sub>6</sub> tag (HHHHHH) (SEQ ID NO: 22) at the 3'-terminus of the coding region was constructed in a transfer vector DNA, *pBacPAK9* (Clontech). Recombinant baculovirus containing the FGF-23 cDNA with the tag sequences was obtained by cotransfection of Sf9 cells with the recombinant *pBacPAK9* and a Bsu36 I-digested expression vector, *BacPAK6* (Clontech). High Five insect cells were infected with the resultant recombinant baculovirus and incubated at 27°C for 72 h in EX-CELL 400 COMPLETE medium (JRH Biosciences).

Paragraph beginning at page 42, line 15, has been amended as follows:

*Preparation of Antisera to FGF-23 by Immunization of Rabbits with an FGF-23 Peptide.* A peptide sequence corresponding to selected contiguous amino acids of the human FGF-23 protein is synthesized and coupled to keyhole limpet hemocyanin (KLH) as described (Harlow and Land, Antibodies: A Laboratory Manual, 1988. Cold Spring Harbor Laboratory, New York, NY). The KLH-coupled peptide is used to immunize rabbits. Antisera are tested for specificity to FGF-23, and for cross-reactivity with other FGF proteins.

Exemplary peptide sequences are:

RRHTRSAEDDSERD (SEQ ID NO: 19) (residues 175-189 of SEQ ID NO:4) and YHLQIHKNGHVDGAPHQ (SEQ ID NO: 20) (residues 51-67 of SEQ ID NO:4).

In the claims:

17. The epitope-bearing portion of claim 15, which comprises amino acids RRHTRSAEDDSERD (SEQ ID NO: 19).

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18. The epitope-bearing portion of claim 15, which comprises amino acids YHLQIHKNGHVDGAPHQ (SEQ ID NO: 20).